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FLUORESCENT DYES AS A MEANS FOR REVEALING ELECTRIC CHARGES ON THE SURFACE AND INSIDE MICROBIAL CELLS

[Following is the translation of an article by A. P. Kononenko and K. I. Kononenko, Kharkov State University imeni A. M. Gor'kogo, published in the Russian-language periodical Biofizika (Biophysics), 14, 1969, pages 187-89. It was submitted on 17 Dec 1966.]

The mechanism by means of which dyes (including fluorescent) are connected to living cells is far from cleared up. It has been proposed that staining sets in on the strength of a chemical affinity of the dyes to specific substances which are found in the cell structures [1-7]. Along with this there is a basis to consider that dyes are bound to cells not only by means of a chemical interaction, but also under the influence of electrostatic attraction between the cell and particles of dye [8, 9].

This article reviews the results of luminescence-microscopic observations, confirming the electrostatic mechanism of interaction of acridine orange and uranin with live microbial cells. In connection with the data obtained the thought emerged concerning the possibility of using these fluorochromes for revealing electric charges on the surface and inside of cells.

The investigations were conducted on vitally fluorochromed bacteria and yeasts (enteric bacilli, staphylococci, Sarcina, and yeast cells of round and oval form) with an ML-2 microscope.

Test 1. Living microorganisms were stained in a neutral aqueous medium with the cation fluorochrome acridine orange (AO) and parallelly, under the same conditions, with the anion dye uranin. In this test a regularly repeated fact is noted: the microbial cells actively sorbed the cation dye AO, but did not absorb the anion dye uranin. The overwhelming majority of cells were stained a green color by AO, however, in each field of vision individuals were encountered which stained red. In preparations which were stained with uranin clearly outlined on a green background were black silhouettes of cells (effect of negative staining). And here the picture was non-uniform. Among the black individuals in each field of vision individuals were noted which luminesced a golden green. In microorganisms with a rod-shaped or elipsoid form, in the event of prolonged contact with dye (one hour and more) the ends are stained red by AO, which testifies to an increased concentration of dye in this area. (Concentration effect of AO luminescence [10, 11]. Under these same conditions in preparations which were stained

with uranin the middle area of the cell acquired a dim-green luminescence, but the ends, conversely, remained black, i.e., did not sorb the dye.

The different relationship of cells to AO and uranin can be explained in the following manner. It is known that microorganisms in an aqueous medium under the influence of an electric current move directionally to the anode and, consequently, their surface charge has a negative sign. As a result of electrostatic attraction the positively charged particles of AO are sorbed by the cell. At the same time the anion dye uranin on the strength of repulsion of like charges is not absorbed by the cell in a neutral medium. Since on a surface with a high degree of curvature the population of charges is usually greater, then around the ends in cells which have an elongated form the intensity of the electric field is higher than in the central area of the cell. This also conditions the active sorption on cellular poles of dye with an opposite sign of charge (AO) and the absence of staining by uranin dye with a like sign of charge.

Test 2. An investigation was made of the interaction of microbial cells with AO and uranin in the presence of salts of strong electrolytes.

In this test it was established that salts, taken in a concentration of 1.7 M for NaCl and 1.3 M for KCl, do not exert a noticeable influence on the intensity and color of luminescence of cells which are found in a solution of AO, but noticeably activate the dyeing properties of uranin. In uranin stained cells the structure is examined easily. For example, in enteric bacilli on a background of a dimly green cytoplasm grains are distinguished distinctly which are luminescing a brilliant green and are located subterminally. In yeast cells spores and vacuoles were apparent. In old cultures cells with a punctate granularity were often detected. Two variants of granularity were noted. Either the cytoplasm luminesced green, but was speckled with black dots, or the cytoplasm remained black, but scattered in it were golden-green dots of various form and size.

As is known, the presence of salts usually promotes staining, although different dyes differ considerably from each other in this feature. The activating influence of salts is explained by the fact that they lower the negative electric potential on the surface of the substrate and reduce the barrier which prevents the approach of annions of dye. In addition to this salts promote the aggregation of dye and reduce processes of desorption [12-14]. As can be seen from the experiment described above, the salts of strong electrolytes at a specific concentration in a solution promote the staining of live microbial cells only with an anion dye - uranin.

Test 3. Live microbial cells were stained with solutions of the same fluorochromes, but at different pH values. By means of

luminescence-microscopic observation it was established that a change of pH in ranges from 10 to 3.5 does not influence the color and intensity of luminescence of live bacteria and yeasts which are found in a solution of AO. Cells of bacteria and yeasts were not stained by solutions of uranin in the pH zones of 10, 9, 5, and 9 /7/. At pH 8.5 and 8 unstained cells predominated, however, almost in every field of vision groups of cells were encountered which luminesced a reddish-brown. Apparently these pH values promote metachromasia of the dye. In pH ranges from 7.5 to 5 microbial cells were not stained by uranin. With a lowering of pH to 4.5 the number of cells with a bright green luminescence increased sharply. We recall that at low pH values uranin changes the sign of the charge and becomes a cation dye. Finally at pH 3.5 all the bacterial cells and more than half the yeast cells were stained. The latter were stained unevenly. The membrane often luminesced a lemon-yellow, and the cytoplasm a golden-green. Round and oval intracellular structures often remained black.

Thus this experiment made it possible to establish that the concentration of hydrogen ions has a definite significance in the staining of cells by the anion fluorochrome uranin, but only in that pH zone where the sign of the charge of the dye is changed. The results of staining dry (but not fixed) smears which were prepared from a microbial suspension did not depend on the sign of the charge on the dye. Both fluorochromes, cation and anion, were sorbed by microbial cells quite intensively. Cells deprived of an aqueous medium lose their surface double electric layer, with which the charge is connected, and transform into an isoelectric state /15/. In this state a selective relationship to dyes with a different sign of charge is not observed.

Thus the luminescence-microscopic observations carried out made it possible to confirm that microbial cells in an overwhelming number carry a negative charge on their surface. However, among the negatively charged cells, cells which are charged positively are detected regularly. The magnitude of the negative charge on cells also is not the same, since with the cation dye AO they are stained not only green, but also a red color. On cells of a cylindrical and ovoid form reinforced electric charges are concentrated on the ends. The value and sign of charge in intracellular structures are apparently variable. For example, usually the spores of yeast cells did not sorb uranin, but in some individuals they luminesced a golden-green. In preparations which were vitally stained with AO the spores inside one ascus often had a different color - two spores gave red and two green fluorescence.

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